sequences, as well as nucleic acid molecules, constructs, and cells including them, and methods employing the sequences, are claimed in U.S. Patent No. 6,242,218 B1. The present application is a continuation of the application that gave rise to the '218 patent, and includes claims to methods of delivering G-CSF to animals, involving introducing cells in which G-CSF gene expression has been activated (e.g., the cells of the '218 patent) into the animals.

The main issue in the Office Action relates to whether the use of cells in which G-CSF expression has been activated in therapeutic methods is enabled. As is discussed further below, applicants respectfully submit that such methods are enabled. In particular, methods for carrying out *ex vivo* gene therapy involving implantation of cells that have been modified to express a therapeutic protein were described in enabling detail well before the priority date of the present application, and these methods could easily be adapted for use in the present invention, simply by replacing the cells used in the prior described methods with those in which G-CSF expression is activated. As a specific example, applicants refer to WO 93/09222 (a copy is enclosed), which describes the production of human growth hormone (hGH) in a mouse in which cells engineered to express hGH had been implanted (Example 10). As is shown in this Example, hGH produced from the implanted cells was detectable in the mouse for a year. Also in this Example, it was shown that cells expressing hGH that were implanted into rabbits produced hGH at relatively constant levels in the rabbits over a period of eleven months.

As another example, applicants note that Example 16 of WO 93/09222 demonstrates the efficacy of cells engineered to express human erythropoietin (EPO) in maintaining hematocrit levels in mice rendered anemic by systematic bleeding. In this Example, nude mice were implanted with rabbit fibroblasts expressing the human EPO gene, and immunoreactive human EPO, distinguishable from endogenous mouse EPO, was readily detectable in the blood of

implanted animals. The animals were subjected to frequent bleeding subsequent to implantation. Bled animals receiving implants of the human EPO-expressing cells exhibited high blood hematocrit levels, as compared to anemic control animals, which showed a drop in hematocrit levels.

In a further example, applicants note that the *ex vivo* gene therapy approach used in the present invention has been the subject of clinical trials in human beings, and has shown promising results. In particular, as is discussed in the enclosed peer-reviewed article from the New England Journal of Medicine (Roth et al., N. Eng. J. Med. 344(23):1735-1742, 2001), cells were obtained from patients suffering from hemophilia and the cells were modified to express Factor VIII. After propagation *in vitro*, the modified cells were harvested and reintroduced into the patients. Increased plasma levels of Factor VIII activity were observed in four of the six patients treated, and this increase was observed to correlate with a decrease in bleeding, a reduction in the use of exogenous Factor VIII, or both. Also, in the patient in whom the highest level of Factor VIII was produced, these clinical changes lasted for approximately 10 months. These observations led the authors to conclude that "this form of gene therapy is feasible in patients with severe hemophilia A" (page 1735).

These examples clearly establish that cells that have been engineered to express a therapeutic protein, such as hGH, EPO, or Factor VIII, can be implanted into an animal (e.g., a human) to produce the protein in the animal. In further support of enablment, applicants also refer to U.S. Patent Nos. 5,968,502 and 6,565,844 (copies are enclosed), which each claim methods for providing a protein to a mammal by introducing into the mammal cells that have been modified such that expression of the protein is activated. The methods of the present claims are similar to those of these patents, but specify the use of the novel and non-obvious G-CSF

promoter sequences that were discovered in the present invention.

Turning now to the specific issues raised in the rejection, applicants note that the Examiner has cited several of the factors from *In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 140 (Fed. Cir. 1988). In particular, the Examiner has commented on the nature of the invention, the amount of direction provided in the specification and the presence or absence of working examples, the state of the prior art, the predictability of the art, and the quantity of experimentation required to practice the invention. Each of these factors and the Examiner's comments with respect to the factors are addressed as follows.

The Nature of the Invention

The Examiner notes that the invention is an *ex vivo* gene therapy method involving transplantation or implantation of cells into animals, including human patients, but does not comment on how these facts relate to the enablement requirement in the present case. Applicants respectfully submit that, in light of the evidence mentioned above, it is clear that the enablement requirement has been met with respect to the present invention. Thus, any considerations as to the nature of the invention are moot.

The Amount of Direction or Guidance in the Specification, and the Presence or Absence of Working Examples

With respect to this basis for the rejection, the Examiner states that the specification does not indicate what levels of G-CSF should be achieved and how such levels are to be maintained during treatment. In reply, applicants note that what is considered to be therapeutic dosages of G-CSF were well known in the art at the time of the present invention and, thus, it was not

necessary to include specific information as to these dosages in the present application. In particular, well before the present invention, G-CSF (also known as NeupoGen® and Filgrastin®) was widely administered to patients, for a number of different ailments. For example, the protein was routinely administered to cancer patients to counteract the negative effects of chemotherapeutic agents on white blood cell count, and standard amounts to administer for this purpose had been established (see, e.g., the enclosed abstracts from PubMed, which were published before the priority date of the present application). Further, applicants note that the examples described above show that expression using the *ex vivo* gene therapy approaches used in the present invention can be used to achieve long lasting, constant expression, if desired. Thus, it would have been a matter of routine optimization to determine the amount of cells to use to achieve therapeutic levels.

The Examiner also questions what types of cells could be used, and notes that different cell types would have different potentials for expression of a recombinant polypeptide, such as G-CSF. Applicants respectfully submit that those of skill in the art certainly could select a particular cell type to use and test it for expression levels. Then, based on the levels detected, could either determine the appropriate amount of cells to implant or determine that another type of cell should be tested. These types of studies are standard and thus do not require undue experimentation.

Further, the Examiner notes that no teaching of actual amelioration of a disease state is set forth in the current application. Applicants submit that such a teaching certainly is not required. As is noted above, the approach used in the present invention had been shown to be effective at delivering therapeutic proteins, and amounts of G-CSF that were established as being therapeutic were known in the art. Thus, applicants respectfully submit that the specification

provides sufficient guidance to enable practice of the present invention, and the fact that working examples of the specifically claimed methods are not present in the application is not relevant to the enablement determination.

The State of the Prior Art, and Predictability or Unpredictability of the Art

The Examiner cites the Orkin and Verma references in support of the enablement rejection, as evidence that the field of gene therapy is unpredictable and that clinical efficacy had not been realized in any gene therapy protocol. These references, expressing a healthy skepticism about gene therapy generally, have nothing to do with whether the present claims are enabled. The studies discussed in the references were done by other workers, using different systems. The fact that the present invention is in a field in which others have experienced problems, using different systems, cannot form the basis of a finding that the present invention is not enabled. Indeed, as is discussed above, *ex vivo* gene therapy methods involving the approach used in the present invention, requiring the implantation or transplantation of cells modified to express a therapeutic product, have been shown to be effective in delivering such products. Thus, the teachings of Orkin and Verma are not relevant to whether the present invention is enabled.

The Quantity of Experimentation

Again referring to the Orkin and Verma references, the Examiner comments that "a very large amount of experimentation of a complex nature would be required to develop any gene therapy protocol to the point of efficacy." In response, applicants again note that the Orkin and Verma references do not address the specific type of *ex vivo* gene therapy approach that is

claimed. Thus, these references cannot be considered to have an impact on whether the present invention is enabled. Moreover, as is discussed in detail above, the general approach used in the present invention had been demonstrated to be effective in therapeutic protein delivery in animal, as well as human, studies. Application of this approach to carrying out the methods of the present invention would involve substituting the cells of the prior methods with the cells of the present invention, which have been modified using novel and non-obvious sequences. Because effective levels of the therapeutic product of the present invention were known (see above), it would at most have required only routine optimization to select a cell line and cell amount to implant in order to achieve therapeutic efficacy. The rejection of the present claims for lack of enablement should therefore be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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